

A Prospective Study of Human Papillomavirus (HPV) Type 16 DNA Detection by Polymerase Chain Reaction and Its Association with Acquisition and Persistence of Other HPV Types

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Human papillomavirus (HPV)-16 causes about half the cases of cervical cancer worldwide and is the focus of HPV vaccine development efforts. Systematic data are lacking as to whether the prevention of HPV-16 could affect the equilibrium of infection with other HPV types and thus alter the predicted impact of vaccination on the occurrence of cervical neoplasia. Therefore, the associations of HPV-16 detection with subsequent acquisition of other HPV types and with the persistence of concomitantly detected HPV types were examined prospectively among 1124 initially cytologically normal women. Preexisting HPV-16 was generally associated with an increased risk for subsequent acquisition of other types. HPV-16 did not affect the persistence of concomitant infections, regardless of type. These findings suggest that the prevention or removal of HPV-16 is not likely to promote the risk of infection with other types, a theoretical concern with current vaccination efforts.

Infection with oncogenic genital human papillomaviruses (HPV) has been established as the main etiologic agent for cervical neoplasia [1]. Among the ≥ 30 genital types of HPV, HPV-16 is the predominant type found in women with a diagnosis of cervical neoplasia, especially invasive cancers [2] and their precursors, high-grade intraepithelial lesions [1]. Although it is much less common in the general population, HPV-16 remains among the most prevalent individual HPV types [3–7]. Because of its strong causal association with about half the cervical cancer cases worldwide [2], current HPV vaccine efforts have focused on HPV-16 infection [8].

About 20%–30% of HPV-infected women harbor multiple HPV types [3, 7, 9]. The direct (viral) and indirect (e.g., immunologic) interactions among multiple HPV infections of the cervix are not understood. Specifically, it is unclear how the equilibrium of other HPV infections might be affected if a type-specific vaccine successfully prevented HPV-16 infection (by prophylactic vaccination) or removed it (by therapeutic vaccination). Particularly, if other cancer-associated types of HPV act differently in the absence of HPV-16 than in its presence,

the expected results of HPV-16 vaccination on cervical neoplasia might be affected.

To investigate the association of HPV-16 with other genital HPV infections, we conducted a prospective study among 1124 originally cytologically normal women whose cervical specimens were collected and tested for HPV DNA by polymerase chain reaction (PCR)-based methods at 2 different times. In this study, we examined the subsequent acquisition, clearance, and persistence of individual types, as well as of various groupings (including phylogenetic clades and cancer-associated types versus low-risk types of HPV), in the presence or absence of HPV-16 DNA at enrollment.

Materials and Methods

Study population. The study was conducted at 7 Kaiser Permanente gynecology or health appraisal clinics in Portland, Oregon. To focus on the target population of prophylactic HPV vaccines, we studied the prospective effects of HPV-16 among initially cytologically normal women. Therefore, the subjects included in this study were selected from a cohort of 17,654 women with normal Pap smear results and no known history of past cervical neoplasia. Details of this cohort are given elsewhere [10]. In brief, these cytologically normal women were recruited between April 1989 and November 1990. At the enrollment visit, in addition to the routine Pap smear, a cervicovaginal lavage specimen was collected and stored at -70°C for HPV testing. The women returned without intervention by study staff, according to the general recommendations of Kaiser Permanente. Thus, this study repre-

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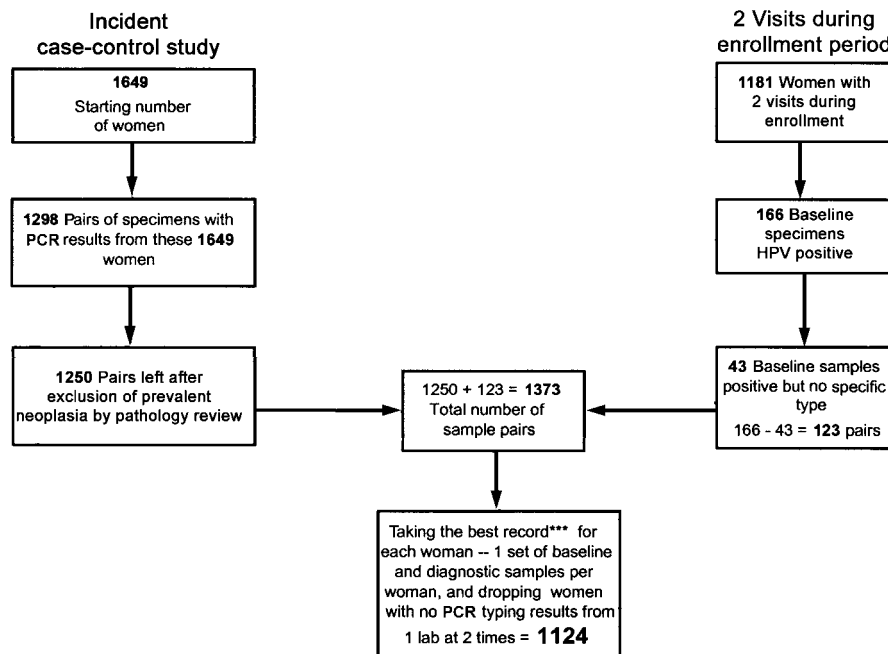


Figure 1. Choice of specimens included in a study of detection of human papillomavirus (HPV)–16, compared with detection of other types. ***Specimen pair with full typing at the same laboratory with longest interval between tests. Final sample of 1124 specimens included 1035 from an incident case-control study and 89 from a “persistence study supplement” of initially HPV-positive women who had 2 clinic visits during enrollment. PCR, polymerase chain reaction.

sented a passive follow-up of women who returned at irregular intervals, with a modal interval of ~1 year.

The 1124 women in this study came from 2 sources within the 17,654-woman cohort. The main population consisted of 1035 women from an incident case-control study nested within the cohort [10]. To achieve greater numbers of initially HPV-infected women for the persistence study, we added 89 women known to be HPV-positive at enrollment who happened to have a second visit during the 18-month recruitment. The 2 sources of study subjects are shown in figure 1.

Women from the incident case-control study were selected from among the 1298 of 1649 participants who had complete pairs of specimens, consisting of the enrollment specimen and a second lavage specimen collected at the time of selection as a case of incident neoplasia or matched control. Forty-eight women were excluded because our pathology review of enrollment and pre-enrollment slides revealed prevalent cervical neoplasia. Of note, second cervical specimens were not collected from women with first Pap abnormalities diagnosed within 9 months of enrollment. Their enrollment specimens were not tested. Such cases were suspected to represent prevalent neoplasia, given that Kaiser Permanente’s policy discouraged Pap tests within 9 months of a previous normal screening.

Despite the lack of closely spaced specimen collections from the incident case-control study, we wished to address the early natural history of women who were HPV-positive at enrollment. Therefore, we added a supplemental group of women who attended the participating clinics more than once during the 18-month enrollment period and whose initial enrollment specimens were HPV DNA–positive ($n = 166$ of 1181 returning twice during enrollment). To study short-

term viral persistence, the selection of subjects intentionally included only women testing HPV-positive initially, with confirmed positivity and adequate HPV typing ($n = 123$; see below).

When we combined the incident case-control and supplemental group, we chose the best specimen pair for each woman, as the one with full typing at a single laboratory with the longest interval between tests. When these final exclusions were made, 1124 women were chosen for the study. Of these, 1035 were in the incident case-control study and 89 were in the supplemental study.

The subjects included 208 women with incident, abnormal cytologic diagnoses at the time of the second specimen collection and 901 women who continued to be cytologically normal. Fifteen women had inadequate diagnoses. We focused the study on HPV DNA testing rather than on cytologic diagnosis of HPV infection. Only 25 of the women with incident cytologic abnormalities had high-grade intraepithelial lesions, and deleting this small fraction of the study population did not affect the conclusions.

Detection of HPV DNA. The 2 paired cervical specimens from each of the 1124 selected women were tested by means of the same PCR-based method [11–13], in 4 collaborating laboratories over the course of the study. All laboratories were masked to any information regarding the subjects. In brief, in all laboratories, cervical lavage specimens were amplified by the L1 consensus primer pair MY09 and MY11. Amplification products were first hybridized with a generic HPV probe mixture, to determine overall positivity. In all laboratories, PCR amplification of a human β -globin gene fragment was used to determine the integrity of the specimens.

Typing methods varied slightly as noted below. The specimens from the incident case-control study were tested at 2 laboratories. The HPV assays for specimens from the first third of the women

in that study were performed at Cetus (Emeryville, CA). The specimens from the remaining women were completed at Albert Einstein College of Medicine (Bronx, NY). Paired specimens were tested in the same laboratory batches, to minimize miscellaneous sources of error.

In both laboratories, type-specific oligonucleotide probes were used, in addition to the generic probes to identify individual HPV types [11–13]. We demonstrated good agreement between the 2 laboratory protocols [11]. The agreement on HPV positivity was 100% among those with single-type infections, whereas, among those with multiple-type infections, the Cetus protocol tended to detect more types. At Cetus, the type-specific probes included HPV-6/-11, -16, -18, -26, -31, -33, -35, -39, -40, -42, -45, -51, -52, -53, -54, -55, -56, -57, -58, -59, -66, -68, -73, and -83, PAPI55, and W13B. Because the probes for HPV-6 and HPV-11 were originally mixed at Cetus, detection of these 2 types was not differentiated, and results were labeled as HPV-6/-11. However, partial retesting data showed that HPV-6 predominated. Sixteen additional type-specific probes were initially included for this study in the assays performed at the laboratory at Albert Einstein College of Medicine, to detect the DNA of HPV-2, -13, -32, -34, -61, -62, -64, -67, -69, -70, and -72, AE2, AE5, AE6, AE7, and AE8 [14]. As discussed below, these additional types were eventually not considered in the data analysis.

HPV testing of specimens for the 89 women in the supplemental group proceeded as follows. The first specimens from the 1181 women who came in twice during enrollment were screened for overall HPV positivity at Roche Biomedical (now Laboratory Corporation of America, Research Triangle Park, NC) with the use of MY09–MY11 consensus primers. Those testing positive ($n = 166$) and a 10% sample of those with initial negative results were retested for positivity and typed at Roche Molecular Systems (Alameda, CA) by means of a reverse dot-blot strip test [15]. The strip test included HPV-6, -11, -16, -18, -26, -31, -33, -35, -39, -40, -42, -45, -51 through -59, -66, -68, -73, and -83, PAPI55, and W13B. Because this study depends on HPV typing, only the 123 specimens that tested positive at both Roche Biomedical and Roche Molecular Systems were included.

HPV testing was not a proven diagnostic modality at the time of this investigation. Therefore, the HPV testing results were not revealed to the clinicians or study participants and did not influence clinical management.

Statistical methods. HPV detection (overall and type-specific) in both initial and second specimens was treated as a binary variable (positive vs. negative) for risk estimation. Analyses were limited to the 27 types that were typed at all laboratories (although the phylogenetically related HPV-6 and -11 were not differentiated in the type-specific analyses). Therefore, we decided to ignore type-specific infections with any of the 16 additional types assayed only at Albert Einstein.

Among the 27 HPV types that were included in the analysis, 4 types, including HPV-26, -42, and -57 and W13B, were not detected in any of the specimens. Thus, the final analytical data set was restricted to 22 types detected, in addition to HPV-16. We analyzed the effects of HPV-16 detection at enrollment on the acquisition and persistence of other non-HPV-16 types. Among women who were initially negative for specific non-HPV-16 types, we studied type-specific acquisition versus continued negativity. Among women who

were initially positive for specific non-HPV-16 types, we studied persistence (positive for a given type at both times) versus clearance.

In addition to the type-specific analyses, we grouped the 22 non-HPV-16 types 2 ways, to achieve greater statistical power. First, we grouped them according to their genetic relatedness (phylogenetic clades), for investigation of the influence of HPV-16 on infection status of these different groups [16, 17] (see also the online listing at <http://hpv-web.lanl.gov>). Second, we grouped the types according to their association with cervical cancer (cancer-associated types vs. low-risk types) [2].

The grouping of phylogenetic clades was based on the L1 sequences of HPV, the region that encodes the major capsid protein [18] and associates with humoral immune responses to HPV infection. The HPV types were categorized into 5 major clades, including clade A9 (the HPV-16 group, including HPV-16, -31, -33, -35, -52, and -58); clade A10 (the HPV-6/-11 group, including HPV-6, -11, and -55); clade A7 (the HPV-18 group, including HPV-18, -39, -45, -59, and -68); clade A3 (the HPV-83 group, including HPV-83 and PAPI55); and clade A6 (the HPV-53 group, including HPV-53, -56, and -66). Four HPV types were not included in the phylogenetic analysis. Of the types we studied and detected, clade A8 includes only HPV-40, clade A11 includes only HPV-73, clade A5 contains only HPV-51, and HPV-54 is not yet assigned to a clade.

In the analyses by phylogenetic clade, the coding had the following logic. In the acquisition analyses, those women who remained negative to all of the types within a clade throughout the study were included as the reference group, whereas those who acquired any of the types within that clade (≥ 1 types) were counted once as “acquisition.” For the persistence analyses, those who had an initial infection with any type within the same clade were included. Women with infections persisting through the follow-up were counted once as “persistence,” regardless of the number of types within that clade that persisted. Only women who lost all the initial infections within that clade were counted (once) as “clearance” in the persistence analyses. The conclusions were not changed by varying the definitions, which affected only multiple infections (e.g., defining persistence as the repeat detection of all types originally found from a clade).

The risk group analysis followed the same logic as the clade analysis. Cancer-associated types other than HPV-16 were defined as types that were present in $\geq 1\%$ of cervical carcinomas in a large international study [2]—that is, HPV-18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, and -68. The low-risk types were defined as HPV-6/-11, -40, -53, -54, -55, -66, -73, and -83 and PAPI55. Women with multiple infections could contribute to both the cancer-associated and low-risk group analyses.

Because of small numbers in each type-specific analysis, we estimated the risks for acquisition and persistence associated with the presence of HPV-16 by use of crude odds ratios (OR) without any adjustment. However, in an attempt to disentangle the effects of HPV-16 from confounding by sexual behavior, we also separately evaluated the risk of acquiring a new type in women reporting having had 0–1 or ≥ 2 sex partners between the 2 visits. Similarly, we evaluated older and younger women separately.

To take the time element into consideration, we used stratification by time period to analyze the persistence of HPV-16 and other types. We plotted the curves for HPV persistence, assuming that each subject was measured halfway through the follow-up time

period when they returned. Use of Kaplan-Meier (life-table) methods instead did not alter the conclusions (data not shown). Women returning during a time interval contributed to the denominator of the persistence rate for that interval. Women contributed to the numerator of the persistence rate for that interval if they still were positive for the type found at enrollment. We examined the persistence of the non-HPV-16 types in 2 major groups, according to the initial presence or absence of HPV-16. Women who did not initially have HPV-16 were further classified hierarchically as having either ≥ 1 cancer-associated versus only low-risk types. Thus, for the graph, each woman contributed to only 1 data point.

Results

HPV infection occurs frequently in younger women; therefore, women selected for this study (median ages, 28 years for the incident case-control group and 23 years for the supplemental group) were younger than the unselected members of the Kaiser Permanente cohort of cytologically normal women (median age, 34 years). The mean follow-up for the 1124 women in the entire study was 25.4 months (range, 8–65 months), with longer follow-up in the acquisition than the persistence analyses (mean, 27.7 vs. 16.6 months). The difference in follow-up time resulted from the dual source of study specimens. As described previously, to assess short-term persistence, we included in our study 89 women who attended the participating clinics more than once during the 18-month enrollment period and whose initial enrollment specimens were HPV DNA-positive.

As presented in table 1, women infected initially with HPV-16 were, in general, more likely to acquire another type of HPV subsequently (overall OR, 1.9; 95% confidence interval [CI], 0.7–4.7). Specifically, an initial infection with HPV-16 significantly increased the risk for the subsequent acquisition of an infection with HPV-18, -39, -45, -59, or -66. On the other hand, none of the women initially infected with HPV-16 acquired an infection with HPV-33, -35, -40, -54, -58, or -83 or PAP155, although some of these types (i.e., HPV-35 and -58) were moderately common in the study population.

No clear pattern was observed for the effect of HPV-16 on type-specific persistence of other types, although the small numbers of concurrent infections for specific combinations were too small to generate any reliable estimates. The risks for persistence of HPV-18, -31, -51, -58, and -83 were increased in the presence of preexisting HPV-16, although none of the risks was statistically significant. In contrast, none of the initial infections with HPV-35, -39, -40, -52, -55, -56, or -73 or PAP155 persisted if an infection with HPV-16 had also been detected initially.

Table 2 shows the effects of the initial HPV-16 infection on the acquisition and persistence of HPV, grouped by genetic relatedness in the sequences of the HPV L1 region. The patterns we observed were not consistent. In clade A9, the risk for acquisition of a non-HPV-16 but phylogenetically related HPV type was nonsignificantly decreased in the initial presence of HPV-16 (OR, 0.3; 95% CI, 0.0–2.4). However, HPV-16 was

strongly associated with an 8-fold risk (OR, 7.9; 95% CI, 3.6–17.2) for subsequent acquisition of HPV-18, -39, -45, -59, or -68 (clade A7). This result was consistent with the significant associations between HPV-16 and subsequent infection with HPV-18, -39, -45, and -59 in table 1.

For comparison with the effect of HPV-16, we assessed the association of some other common types of HPV with the acquisition of additional types in their same phylogenetic clades (table 2). Therefore, in clades A9, A7, and A6, respectively, we also examined the effects of HPV-31, -18, and -53 on the subsequent infection status of phylogenetically related types. In contrast to the result seen for HPV-16 and other members of clade A9, HPV-31 was positively, albeit nonsignificantly, associated with acquisition of other A9 types (OR, 1.6; 95% CI, 0.4–7.0). Similarly, HPV-18, a member of clade A7, was weakly associated with the acquisition of the other members of the same clade (OR, 2.0; 95% CI, 0.3–15.6). Moreover, in clade A6, infection with HPV-53, just like HPV-16, was associated with an ~ 3 -fold risk (OR, 2.6; 95% CI, 0.8–9.0) for a subsequent acquisition of any of the types in this clade.

None of the risks for the persistence of HPV in the various clades was significantly altered in the presence or absence of HPV-16, or other types, at enrollment.

In addition to the effects on HPV infection status in different clades, we investigated the impact of HPV-16 infection on acquisition and persistence of non-HPV-16 types grouped by their strength of association with cervical cancer (i.e., cancer-associated types vs. low-risk types) [2]. In table 3, the risk for acquisition of either a cancer-associated type (OR, 2.6; 95% CI, 1.2–5.7) or a low-risk type (OR, 2.7; 95% CI, 1.1–6.7) was increased in the presence of an initial HPV-16 infection. The risk for the persistence of a cancer-associated or a low-risk type was unchanged by initial presence of HPV-16.

Figure 2 shows the probability of the initial infection with non-16 HPV persisting over the time of follow-up in the initial presence and absence of HPV-16, as well as the persistence of HPV-16 itself. HPV-16 persisted longer than the other type groups. HPV-16 persistence was unchanged in the women who concurrently had other types as well (data not shown). Moreover, HPV-16 appeared to have little effect on the persistence of other types detected initially. It is noteworthy that the persistence of cancer-associated types was similar to that of low-risk types, when HPV-16 was not detected initially.

Relatively few women were examined at intervals of >27 months. Anecdotally, among women with HPV-16 initially who returned later than 27 months, 3 of 7 remained positive for HPV-16. The 2 women with longest HPV-16 persistence, 42 and 47 months, had histologically confirmed high-grade lesions.

In ancillary analyses, we explored whether age or sexual behavior might explain the association of HPV-16 initial detection with increased risk of acquisition of other HPV types. We divided women into older versus younger (median age, 27 years). Overall, HPV acquisition was more likely for younger (20.3%

Table 1. Risks for acquisition and persistence of individual human papillomavirus (HPV) type associated with presence of HPV-16 at enrollment.

Risk for HPV type, previous status for HPV-16	No. remaining negative (−/−)	Acquisition (−/+)		Clearance (+/-), no.	Persistence (+/+)	
		No.	OR (95% CI)		No.	OR (95% CI)
HPV-6/-11						
HPV-16−	1030	16	1.0 (—)	12	5	1.0 (—)
HPV-16+	52	3	3.7 (1.0–13.1)	5	1	0.5 (0.0–5.2)
HPV-18						
HPV-16−	1039	8	1.0 (—)	9	7	1.0 (—)
HPV-16+	54	3	7.2 (1.9–28.0)	1	3	3.9 (0.3–45.6)
HPV-31						
HPV-16−	1023	20	1.0 (—)	9	11	1.0 (—)
HPV-16+	57	1	0.9 (0.1–6.8)	1	2	1.6 (0.1–21.1)
HPV-33						
HPV-16−	1048	5	1.0 (—)	3	7	1.0 (—)
HPV-16+	59	0	0 (—)	1	1	0.4 (0.0–9.4)
HPV-35						
HPV-16−	1046	10	1.0 (—)	6	1	1.0 (—)
HPV-16+	60	0	0 (—)	1	0	0 (NA)
HPV-39						
HPV-16−	1039	11	1.0 (—)	10	3	1.0 (—)
HPV-16+	55	4	6.9 (2.1–22.3)	2	0	0 (NA)
HPV-40						
HPV-16−	1052	3	1.0 (—)	7	1	1.0 (—)
HPV-16+	59	0	0 (—)	2	0	0 (NA)
HPV-45						
HPV-16−	1046	3	1.0 (—)	11	3	1.0 (—)
HPV-16+	58	2	12.0 (2.0–73.4)	0	1	∞ (NA)
HPV-51						
HPV-16−	1005	26	1.0 (—)	25	7	1.0 (—)
HPV-16+	55	3	2.1 (0.6–7.2)	2	1	1.8 (0.1–22.7)
HPV-52						
HPV-16−	1027	17	1.0 (—)	12	7	1.0 (—)
HPV-16+	58	2	2.1 (0.5–9.2)	1	0	0 (NA)
HPV-53						
HPV-16−	992	30	1.0 (—)	29	12	1.0 (—)
HPV-16+	51	3	1.9 (0.6–6.6)	5	2	1.0 (0.2–5.7)
HPV-54						
HPV-16−	1038	8	1.0 (—)	9	8	1.0 (—)
HPV-16+	56	0	0 (—)	4	1	0.3 (0.0–3.1)
HPV-55						
HPV-16−	1054	5	1.0 (—)	4	0	1.0 (—)
HPV-16+	60	1	3.5 (0.4–30.5)	0	0	NA (NA)
HPV-56						
HPV-16−	1035	17	1.0 (—)	3	8	1.0 (—)
HPV-16+	56	2	2.2 (0.5–9.6)	3	0	0 (NA)
HPV-58						
HPV-16−	1028	19	1.0 (—)	12	4	1.0 (—)
HPV-16+	56	0	0 (—)	3	2	2.0 (0.2–16.6)
HPV-59						
HPV-16−	1046	9	1.0 (—)	6	2	1.0 (—)
HPV-16+	54	3	6.5 (1.7–24.5)	3	1	1.0 (0.1–16.0)
HPV-66						
HPV-16−	1041	12	1.0 (—)	9	1	1.0 (—)
HPV-16+	57	3	4.6 (1.3–16.6)	0	1	∞ (NA)
HPV-68						
HPV-16−	1052	5	1.0 (—)	3	3	1.0
HPV-16+	58	1	3.6 (0.4–31.6)	1	1	1.0 (0.0–24.5)
HPV-73						
HPV-16−	1046	8	1.0 (—)	8	1	1.0 (—)
HPV-16+	57	2	4.6 (1.0–22.1)	2	0	0 (NA)
HPV-83						
HPV-16−	1049	5	1.0 (—)	7	2	1.0 (—)
HPV-16+	59	0	0 (—)	1	1	3.5 (0.1–84.7)
PAP 155						
HPV-16−	1039	8	1.0 (—)	11	5	1.0 (—)
HPV-16+	58	0	0 (—)	3	0	0 (NA)

NOTE. CI, confidence interval; NA, not assessable; OR, odds ratio; +, positive; −, negative. Pluses and minuses in parentheses refer to status for particular HPV type at enrollment/at subsequent testing.

Table 2. Risks for acquisition and persistence of human papillomavirus (HPV), by phylogenetic clade in presence or absence of selected types of HPV.

Phylogenetic clade, risk for HPV type, previous status for selected HPV type	No. remaining negative (−/−)	Acquisition (−/+)		Clearance (+/-), no.	Persistence (+/+)	
		No.	OR (95% CI)		No.	OR (95% CI)
A9						
HPV-31, -33, -35, -52, -58						
HPV-16−	937	58	1.0 (—)	38	30	1.0 (—)
HPV-16+	49	1	0.3 (0.0–2.4)	7	4	0.7 (0.2–2.7)
HPV-16, -33, -35, -52, -58						
HPV-31−	922	73	1.0 (—)	46	60	1.0 (—)
HPV-31+	16	2	1.6 (0.4–7.0)	3	2	0.5 (0.1–3.2)
A10						
HPV-6/-11, -55						
HPV-16−	1022	21	1.0	15	5	1.0
HPV-16+	52	3	2.8 (0.8–9.7)	5	1	0.6 (0.1–6.4)
A7						
HPV-18, -39, -45, -59, -68						
HPV-16−	978	31	1.0 (—)	36	18	1.0 (—)
HPV-16+	40	10	7.9 (3.6–17.2)	6	5	1.7 (0.4–6.2)
HPV-39, -45, -59, -68						
HPV-18−	1027	32	1.0 (—)	32	13	1.0 (—)
HPV-18+	16	1	2.0 0.3–15.6()	2	1	1.2 (0.1–14.8)
A3						
HPV-83, PAP155						
HPV-16−	1025	13	1.0 (NA)	18	7	1.0 (—)
HPV-16+	56	0	0 (NA)	4	1	0.6 (0.1–6.8)
A6						
HPV-53, -56, -66						
HPV-16−	957	50	1.0 (—)	35	21	1.0 (—)
HPV-16+	45	6	2.6 (1.0–6.3)	7	3	0.7 (0.2–3.1)
HPV-56, -66						
HPV-53−	1028	30	1.0 (—)	9	9	1.0 (—)
HPV-53+	39	3	2.6 (0.8–9.0)	5	1	0.2 (0.0–2.1)

NOTE. CI, confidence interval; NA, not assessable; OR, odds ratio; +, positive; −, negative. Pluses and minuses in parentheses refer to status for particular HPV type at enrollment/status at subsequent testing.

for any type) than for older women (5.5%). The 9 older women who were HPV-16-positive at enrollment resembled younger women in that their acquisition rate was 22.2%. Therefore, the relative increase in acquisition associated with the presence of HPV-16 was seen among the older women (OR, 5.2; 95% CI, 1.0–26.6) but not among the younger women (OR, 1.0; 95% CI, 0.3–3.0). The lack of effect of HPV on the persistence of other types was seen in both age strata, although viral persistence of any type other than HPV-16 was slightly more likely among the older women (46.3% vs. 37.3%, not statistically significant).

With regard to sexual behavior, we divided women according to the number of sex partners they reported having between enrollment and the collection of the second specimen (when a questionnaire was administered). For 104 women, including all the women in the supplemental group, this information was missing. The presence of HPV-16 at baseline was strongly and significantly predictive of subsequent acquisition of both high-risk and low-risk groups among women reporting 0 or 1 partner (OR, 5.6 and 6.3, respectively). The associations resulted from very low acquisition rates among HPV-negative women with 0–1 partner (the low-risk group analogous to the older women

in the age analysis), not from especially high acquisition among HPV-positive women with 0–1 partner. Women reporting ≥ 2 partners since enrollment had much higher acquisition rates than did those with 0–1 partner, regardless of HPV-16 status (analogous to the younger women in the age analysis). Accordingly, among women with more partners, the relative risks of HPV acquisition associated with HPV-16 detection were weak and nonsignificant.

As a final point, we investigated the impact of the clearance of HPV-16 on the acquisition of other types. We found that, among the 61 women with HPV-16 initially, those without HPV-16 in the second specimen had the same risk of acquisition of another type of HPV at that second time as did those who remained HPV-16-positive (OR, 1.0; 95% CI, 0.3–3.1).

Discussion

In this large-scale prospective study, we examined the association of an initial infection with HPV-16, the most common cancer-associated type [2], with the subsequent acquisition of another type and with the subsequent persistence of other HPV types detected concomitantly. We found that an infection with

Table 3. Risks for acquisition and persistence of cancer-associated and low-risk types of human papillomavirus (HPV) associated with presence of HPV-16 at enrollment.

Risk for HPV types, previous status for HPV-16	No. remaining negative (−/−)	Acquisition (−/+)		Clearance (+/-), no.	Persistence (+/+)	
		No.	OR (95% CI)		No.	OR (95% CI)
Cancer-associated types ^a						
HPV-16−	825	94	1.0 (—)	84	60	1.0 (—)
HPV-16+	30	9	2.6 (1.2–5.7)	14	8	0.8 (0.3–2.0)
Low-risk types ^b						
HPV-16−	904	57	1.0 (—)	69	33	1.0 (—)
HPV-16+	35	6	2.7 (1.1–6.7)	15	5	0.7 (0.2–2.1)

NOTE. CI, confidence interval; –, negative; OR, odds ratio; +, positive. Pluses and minuses in parentheses refer to status for particular HPV type at enrollment/status at subsequent testing.

^a HPV-18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, and -68.

^b HPV-6, -11, -40, -53, -54, -55, -66, -73, and -83 and PAP155.

HPV-16 is associated with a generally increased risk of subsequent HPV acquisition but does not affect the subsequent persistence of concomitant HPV infections.

Despite the large size of the study, specific type combinations were uncommon, limiting statistical power, particularly for the examination of persistence. Nor could we look at certain more subtle points of interest. For example, our findings suggested that the persistence or clearance of HPV-16 did not affect the risk of acquiring another new HPV type. To gain statistical power, we created groupings based on phylogenetic relatedness and known associations with risk of cancer. However, forcing possibly artificial groupings of clades and risk categories might have obscured details of type-type interactions. Still, the broad patterns seemed clear.

The generally increased risks for acquisition of another type of HPV among the women who had a preexisting infection with HPV-16 suggest the common mode of transmission. It is probable that a previous infection with HPV-16 is merely a marker of sexual exposure that, in turn, represents more exposure to other types of HPV. HPV-16 detection increased the risk for the acquisition for other HPV types among women with 0–1 partner and among older women, both low-risk groups. Because we could not differentiate when a single sex partner during follow-up was a new one, we could not rule out the possibility that HPV-16 at enrollment was somehow associated with “high-risk” new sex partners, which could also produce a positive association. Alternatively, the detection of HPV-16 (or any other type) may imply that the woman is immunologically susceptible to HPV infections generally, although the age and sex partner data argue for a behavioral explanation.

The observations regarding type-specific HPV acquisition were reflected in the grouped analysis by phylogenetic clades. The risk for the acquisition of a particular HPV type in most phylogenetic clades was elevated in the presence of a preexisting infection with HPV-16.

As an exception, the risk of acquiring a new infection with non-HPV-16 types in clade A9 (HPV-31, -33, -35, -52, or -58) was not increased among those who already had an infection with HPV-16 (OR, 0.3; 95% CI, 0.0–2.4), which belongs to that

clade. Before any biologic interpretations are made, chance could explain the singular observations for the combination of HPV-16 and other types within clade A9. With that definite caveat, there is a theoretical possibility that a previous infection with HPV-16 could continuously elicit immunologic responses (most likely cell-mediated responses) that reduce the subsequent point prevalence of HPV types that are genetically most related to HPV-16. Such protective cross-reactivity could alter the impact of an HPV-16 vaccine. However, arguing against cross-protection, we observed that HPV-31 was associated with a slightly increased risk of infection with related types in clade A9, thereby leaving the HPV-16 finding isolated and unexplained.

Regarding viral persistence, we found that an initial infection with HPV-16 did not affect the subsequent persistence of the concomitant infection with other types detected initially. Al-

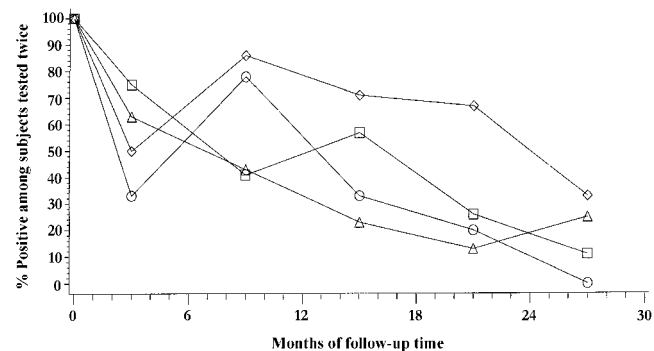


Figure 2. Persistence of human papillomavirus (HPV) types over time. Persistence (still positive for type initially demonstrated at enrollment) was plotted in 4 mutually exclusive groups of initial HPV DNA detection: HPV-16 alone (◇, $n = 29$), all other types in presence of HPV-16 (○, $n = 32$), cancer-associated types in absence of HPV-16 (□, $n = 144$), and low-risk types in absence of HPV-16 or other cancer-associated types (△, $n = 56$). Women returning during each 6-month period were plotted at that period's midpoint. Plotting was truncated at 27-month interval because later intervals had unstable estimates because of small numbers. Cancer-associated types included HPV-18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, and -68. Low-risk types included HPV-6, -11, -40, -53, -54, -55, -66, -73, and -83 and PAP155.

though our study population was large and included additional HPV-positive subjects, <300 had any type of HPV infection initially, resulting in unstable estimates for type-specific persistence analyses. Despite limited numbers, the general pattern clearly suggested a null effect on persistence. Analyses by phylogenetic clade or by association with cervical cancer supported our conclusion of unaltered risks for the persistence of a non-16 HPV in the presence of a preexisting infection with HPV-16.

The time analysis shown in figure 2 indicated that persistence of other types is not noticeably affected by the initial presence of HPV-16 at any given point during the follow-up. The longer persistence of HPV-16, compared with other types (cancer-associated and low-risk alike), suggests that the earlier findings of a higher percentage of persistence for cancer-associated types may be influenced predominantly by HPV-16 [13], although one recent study did not reach this conclusion [9].

In summary, our acquisition data suggest that infection with HPV-16 is associated with an increased risk of subsequent acquisition of another type of HPV, through sexual behavior- or immunology-related mechanisms. The findings (with the possible exception of the data regarding clade A9) do not support a field effect hypothesis that the first established HPV infection on the cervix will reduce infection with other genetically related HPV types. The persistence data imply that concomitant infections with HPV-16 and other types, once established on the cervix, will not affect each other subsequently. Overall, our findings suggest that the prevention or removal of HPV-16 is not likely to promote the risk of infection with other types, a theoretical concern with current vaccination efforts [8, 18]. On the basis of particularly the persistence data, it appears that HPV types tend to act as independent sexually transmitted diseases.

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